

In response to the article entitled “Prevalence of different types of gallstone in the patients with cholelithiasis at Kathmandu Medical College” by Pradhan SB, Joshi MR and Vaidya A published in KUMJ 2009 Vol 7, No. 3, Issue 25, 268-71

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Dear editor,

Cholelithiasis or gallbladder stones are one of the major surgical problems. The chemical composition of gallstones is essential to study aetiopathogenesis of gallstone disease. Although the gallstones vary in chemical composition, they generally contain a mixture of cholesterol, bilirubin, calcium, and mucoproteins¹. Several methods are available for the analysis of calculi including optical crystallography, X-ray diffraction, electron diffraction, infrared spectroscopy and chemical analyses. We have read with interest on recent reports of the chemical analysis of gallstone in the article by Pradhan SB *et al*² entitled “Prevalence of different types of gallstone in the patients with cholelithiasis at Kathmandu medical college, Nepal” published in KUMJ 2009 vol 7 No 3 issue 27 page 268-271. Though it was a nice attempt of the authors to analyze the gallstone chemically for the first time in Nepal, there is, however, a fundamental flaw in this study that we wish to bring to general attention.

Interestingly, the authors had dissolved 500 mg grinded powder of gallstone in 5 mL of distilled water and filtrate was used to analyze cholesterol, bilirubin, calcium and phosphorus. By the best of our knowledge cholesterol being insoluble in water cannot be extracted from water. Standard text books have mentioned that cholesterol should be extracted using chloroform or other fat solvent from the stone before its assays^{3,4}. Several studies had used the chloroform extraction procedure to analyze cholesterol in gallstone^{5,6}. Study conducted in India by Pundir CS *et al*^{7,8} and Jarari AM *et al*⁹ also have used chloroform for the extraction of cholesterol from gallstone. Similarly, bilirubin is extracted in methanol and, for the estimation of calcium and phosphorus, acid extraction is necessary. Overall these extraction procedures are laborious.

We also tried to analyse gallstone by procedure as mentioned by Pradhan SB *et al*² in our laboratory to

check whether it really works but it failed. We have analyzed five gallstones by chloroform extraction procedure. After grinding stone, 30 mg of the powder was dissolved in 3 mL chloroform in a test tube^{7,10}. The tube was kept in boiling water bath for 2 minutes. The stone solution thus obtained was used for determination of cholesterol by Libermann-Burchard reaction¹¹ and enzymatic cholesterol method¹². Cholesterol was detected in all five stone by both reactions. However, both reactions failed to detect cholesterol by the procedure mentioned by Pradhan SB *et al*.

Overall, it is questionable whether quantification by so-called distilled water extraction method really works for analyzing gallstone and adds value to the diagnostic investigation!

References

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Author's Reply

Dear reader,

First of all I would like to thank you for the appreciation of my attempt to analyse gallstones and your concerns and the opportunity to let me explain more on my study. I agree to the fact that any scientific study should be reproducible. We analysed the stones as mentioned in our article¹⁻⁷. The ones showing negative results by our method were also extracted by acidified methanol chloroform mixture (1:1v/v) and analysed by enzymatic method. I would thus like to request you to check your procedure again. Lastly, I always welcome comments from my fellow readers, as no science can be complete till it is fully questioned, analysed and lastly accepted.

Thank you

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